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THE USE OF CANOLA, PEA AND FLAX FRACTIONS IN AQUAFEEDS

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Use of Canola, Pea and Soy fractions in Aquafeeds

Final Report
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Summary

The objective of this project was to determine the effect of inclusion rate of pea, canola and soybean products on nutrient digestibility and growth performance of rainbow trout. This project experienced a major delay due to an equipment failure at the Prairie Aquaculture Research Centre in 2007 that delayed the project by 2 years. Due to this delay, it was agreed to complete the growth studies in Milestones 4 and 5 and to drop the intestinal inflammation studies in Milestone 3 due to time constraints.

The major results of these studies are to demonstrate that pea and canola fractions do not affect the growth and intestinal function of rainbow trout in the same manner as soybean meal. Specifically:

1) Pea protein concentrate and canola protein concentrate have no effect on the digestibility of dry matter, energy or protein at levels up to 40% in the diet. In contrast, soybean meal significantly reduces the digestibility of these dietary components.
2) Pea meal and pea protein concentrate can be fed at levels up to 30% of the diet without affecting the growth performance or feed intake of rainbow trout.
3) Canola protein concentrate can be fed at levels up to 30% of the diet without affecting growth performance or feed intake of rainbow trout. However, canola meal reduces the feed intake and growth of rainbow trout at levels less than 7.5% inclusion rate.
4) Soybean meal and soy protein concentrate significantly reduce fish growth and feed intake at levels above 15% of the diet.

These results are being directly shared with researchers in Norway who will replicate these studies in Atlantic salmon. This work supports the use of pea and canola protein concentrates as replacements for fish meal in salmonid diets.

Introduction

Replacing fishmeal with plant proteins has proven a difficult nut to crack. Soybeans are the principal source of protein in diets fed to terrestrial farm animals accounting for approximately 75% of protein fed in animal diets. The natural assumption is that soybeans should therefore easily and economically replace fish meal in salmonid diets. Soybean meal contains approximately 48% crude protein and with the addition of methionine has an excellent balance of essential amino acids. However, a host of studies have reported that inclusion rates of greater than 20-30% soybean meal results in decreased weight gains and increased conversion rates. These effects have been attributed to the presence of antinutritional factors (ANFs) present in soybean meal. Heat labile ANFs, including trypsin inhibitor and lectins, can be eliminated or reduced by a heat treatment during the normal processing of SBM. Heat stable ANFs present in SBM include non-starch polysaccharides (NSP), saponins, phytate, phytoestrogens and protein antigens. These factors must be removed from soybean meal by fractionation or inactivated in some other way. Soybean protein concentrates and isolates are lower in

heat stable ANFs and may be used at higher inclusion rates than soybean meal. However, they are cost prohibitive ($1500-3000 per tonne). Clearly soybeans are not final solution to replacing fish meal in salmonid diets.

Field Peas

Aquafeeds may contain high levels of digestible protein of up to 45% in the case of salmonid fish. Fish meal provides a palatable, nutrient dense source of protein containing from 60-75% crude protein with an amino acid balance that closely meets the requirements of the fish. In contrast, field peas contain approximately 21% crude protein which makes their use in high protein salmonid diets unfeasible. However, peas are low in antinutritional factors compared to soybean meal suggesting that they have potential as an ingredient in salmonid diets if their protein levels can be increases. Air classification of peas is economical and results in pea protein concentrate with a protein content of up to 60%.

Canola

Canola is the name given to selected varieties of rapeseed which are low in glucosinolates and erucic acid. Canola meal contains only 35% crude protein, 12% crude fibre and up to 4% phytic acid, thus, limiting its use in aquafeeds. Aqueous extraction of canola protein to produce canola protein concentrate results in a product with approximately 70% crude protein, 2.8% crude fibre and 0% phytate. Furthermore, canola protein has a high biological value compared to other protein sources. For example, canola protein concentrate has a protein efficiency ratio of 3.29 compared with 1.60 for soyabean protein concentrate and 3.13 for casein.

Project Objective

The overall objective of this project was to determine the effect of inclusion rate of soybean meal, soybean protein concentrate, pea meal, pea protein concentrate, canola meal and canola protein concentrate on nutrient digestibility and growth performance of rainbow trout.

Milestones

Milestone 1 Soy, Pea and Canola Products

Soybean meal was obtained from the Federated Cooperative feedmill in Saskatoon. Soyprotein concentrate (Soycomil P) was obtained from ADM Canada. Field peas were obtained from the University of Saskatchewan Crop Development Centre (CDC Mozart) and pea protein concentrate was obtained from Parrheim Foods Saskatoon. Canola meal was obtained from the Northwest Terminal, Unity SK. One tonne lots of these ingredients were obtained and stored in a secure facility. Canola protein concentrate was obtained

from MCN Bioproducts, Saskatoon SK. It was only available in small amounts so various lots have been used during this research.

Milestone 2. Extrusion processing of crop fractions

No suitable extruder could be located so the pea, canola and soybean ingredients were analyzed as is. The results of these analyses are shown in Tables 1 and 2.

Table 1. Nutrient composition of 6 ingredients used in this project

Component	SBM	SPC	PM	PPC	CM	CPC
Dry matter (%)	92.2	94.97	90.12	93.85	91.82	95.43
Crude protein (%)	52.75	72.06	24.77	49.6	39.92	66.65
Gross energy (kcal / g)	4583	4736.39	4312.35	4698.14	4486.38	5310.51
Ash (%)	7.25	6.16	2.69	4.77	11.54	5.39
Lipid (%)	3.55	1.05	2.49	4.86	5.89	7.42
ADF (%)	6.04	13.13	7.74	7.62	11.2	1.5
NDF (%)	9.18	29.45	12.73	13.71	5.8	1.1

Amino acid analysis % DM basis	SBM	SPC	PM	PPC	CM	CPC
Essential AA						
Arginine	3.8	3.36	2.17	4.3	2.39	4.04
Cystine	0.76	0.69	0.34	0.64	0.97	1.04
Histidine	1.4	1.29	0.59	1.2	1.12	1.93
Isoleucine	2.37	2.39	0.99	1.97	1.55	2.87
Leucine	4.01	4.64	1.73	3.51	2.78	5.12
Lysine	3.26	3.16	1.78	3.62	2.23	3.25
Met & cys	1.47	1.74	0.57	1.09	1.8	2.42
Methionine	0.72	1.06	0.23	0.45	0.83	1.37
Phenylalanine	2.64	2.63	1.16	2.39	1.57	2.94
Threonine	2.04	2.07	0.9	1.83	1.73	2.9
Valine	2.48	2.68	1.12	2.22	1.98	3.57
Non-essential Amino acids						
Alanine	2.25	2.81	1.02	2.05	1.74	3.07
Aspartic acid	5.96	5.31	2.82	5.57	2.8	5.16
Glutamic acid	9.53	9.67	4.03	7.97	7.06	11.07
Glycine	2.2	2.47	1.04	2.05	1.98	3.49
Proline	2.54	2.99	0.95	1.95	2.64	3.89
Serine	2.64	2.51	1.16	2.42	1.71	2.87
Total w/o NH3	48.61	49.74	22.03	44.12	35.07	58.59
Ammonia	1.04	1.13	0.42	0.81	0.74	1.12
Total	49.66	50.87	22.45	44.93	35.81	59.71

Table 2. Antinutritional factors present in the 6 ingredients.

Antinutritional Factor	SBM	SPC	PM	PPC	CM	CPC
Glucosinolates (umoles/g)						
3-butenyl	0	0	0	0	2.04	0.63
4-pentenyl	0	0	0	0	0.15	0.08
2-OH-3-butenyl	0	0	0	0	4.92	0.54
CH3-thiobutenyl	0	0	0	0	0.16	0
Phenylethyl	0	0	0	0	0.1	0.06
3-CH3-indolyl	0	0	0	0	0.26	0
4-OH-3-CH3-indolyl	0	0	0	0	1.05	0.15
Total aliphatics	0	0	0	0	7.14	1.25
Tannins	0.84	0.54	0.49	0.76	1.06	0.62
Isoflavones (mg/g)						
Daidzin	2.1	0.01	0	0	0	0
Glycitin	0.35	0	0	0.02	0	0
Genistin	0	0	0	0	0	0
Daidzein	0	0	0	0	0	0
Glycitein	0	0	0	0	0	0
Genistein	0	0	0	0	0	0
7-Hydroxy-4- Methoxyisoflavone	0	0	0	0	0	0
5,7-Dihydroxy-4- Methoxyisoflavone	0	0	0	0	0	0

Milestone 3. Digestibility studies

Introduction

The purpose of these studies was to measure the effect of inclusion rate of ingredients on the digestibility of dry matter, energy and protein in rainbow trout. When we perform a digestibility study, we add the ingredient to the diet at 30% inclusion level and measure the digestibility of nutrients. We performed such an analysis in Experiment 1. However, digestibility of nutrients may be different at different inclusion levels. We therefore measured the digestibility of corn gluten meal, canola protein concentrate, pea protein concentrate, soybean meal, fish meal and wheat at 0, 10, 20 30 and 40% inclusion levels in Experiment 2.

Experiment 1

Objective

To determine the digestibility of all ingredients to be used in diet formulations using a standard 30% inclusion rate.

Materials and methods

The experimental ingredients for this trial consisted of the following: corn gluten meal, canola meal, canola protein concentrate, pea meal, pea protein concentrate, soybean meal, soy protein concentrate, fish meal and wheat.

Fish Management

Rainbow trout were maintained in 350 L tanks that were part of a recirculating system using biological filtration. Water temperature was maintained at 15 ± 1° C. Dissolved oxygen, nitrate, nitrite, ammonia and pH were monitored regularly. Photoperiod was a 14 h light/10 h dark cycle. Seventeen (276.5g; average weight) fish per tank were utilized with five replicates per treatment. The fish were maintained in accordance with the guidelines of the Canadian Council on Animal Care (CCAC 1984).

Digestibility Determination

Apparent digestibility coefficients (ADC, %) were measured using an indirect method with diets containing 1% celite as a nonabsorbable indicator. A reference diet (Table 1) was formulated according to Bureau and Cho (1994). The experimental diets were formulated using 70% of the reference diet with 30% of the experimental ingredient (dry matter basis). The diets were cold extruded, dried in a forced air oven (55°C, 12 h), chopped and screened to obtain the appropriate pellet size. The fish were adapted to the experimental diets for five days and fecal material collected over a seven day period using a settling column which separated the fecal material from the effluent water. Following collection, feces were centrifuged (5000 X g, 15 min), frozen and freeze dried.

Laboratory Analysis

Experimental diets and fecal material were analyzed for moisture (AOAC 1990, method no. 934.01), energy (oxygen bomb calorimetry; Parr Adiabatic Calorimeter, Model 1200), and acid ether extract (AOAC 1995, method no. 954.02). Nitrogen content was determined by combustion method (AOAC 1995). Protein was estimated by multiplying nitrogen content by 6.25. Determination of acid insoluble ash was performed according to the following procedure. Samples (quadruple replicates) were charred (250°C, 18 h), followed by a gradual increase in temperature to 500°C (3 h), and then ashed (48 hours). Four mL of 4N HCl was added and the samples were heated at 120°C for a minimum of one hour. Samples were then centrifuged (3000 x g, 10 min) followed by aspiration of the supernatant. Five mL of water was added, vortexed, centrifuged and aspirated two times. The samples were then dried overnight (80°C) followed by ashing (500°C, 24 h).

Digestibility Calculations

The ADC (%) for the reference and experimental diets were calculated as follows:

$$ADC = 1 - (F/D \times Di/Fi)$$

Where:
D = % nutrient in the diet (dry matter (DM) basis)
F = % nutrient in the feces (DM basis)
Di = % indicator in the diet (DM basis)
Fi = % indicator in the feces (DM basis)

The ADC of the test ingredient was calculated as follows (Sugiura et al. 1998):

$$ADCI = ADCT + ((1-s) DR/s DI) (ADCT - ADCR)$$

Where:
ADCI = Apparent digestibility coefficient of test ingredient
ADCT = Apparent digestibility coefficient of test diet
ADCR = Apparent digestibility coefficient of the reference diet
DR = % nutrient (or kJ/g gross energy) of the reference diet mash (DM basis)
DI = % nutrient (or kJ/g gross energy) of the test ingredient (DM basis)
s = Proportion of test ingredient in test diet mash (DM basis)

Analysis of the results used the General Linear Model procedure of SAS (SAS Version 8.0). Mean values were separated using the Student-Newman-Keuls test with the accepted level of significance at $P<0.05$.

Results

The chemical analysis and Apparent Digestibility Coefficients for the test ingredients are shown in Tables 2 and 3 respectively. We have not completed the protein and amino acid analysis and these results are not shown. The results of this trial agree closely with previous studies at the University of Saskatchewan.

Table 3. Composition of reference diet utilized in digestibility study

Ingredient	Inclusion (g kg^{-1})
Fishmeal[a]	300
Soybean meal	170
Corn gluten meal	130
Wheat flour	280
Vit./Min. premix[b]	10
Celite[c]	10
Fishoil[d]	100
(Total)	1000

[a]South American Aquagrade; EWOS Canada Ltd.
[b]The vitamin/mineral premix was a commercial premix (EWOS; closed formulation) formulated to meet the requirements of juvenile rainbow trout.
[c]Celite 545, <125µm; Celite Corporation, World Minerals Co., Lompoc, CA, USA
[d]Mixed variety fish oil; EWOS Canada Ltd.

Table 4. Chemical composition of test ingredients (DM basis).

Ingredient	Dry Matter (%)	Gross Energy (MJ/kg)	Crude Protein (%)
Corn Gluten Meal	92.16	22.75	70.52
Canola Meal	91.82	19.93	39.92
Canola protein Concentrate	93.47	20.07	69.42
Fish meal	94.32	21.39	76.17
Soybean meal	92.20	19.27	52.75
Soy Protein Concentrate	94.47	19.84	72.06
Pea Meal	90.12	18.61	24.77
Pea protein concentrate	91.51	20.41	49.60
Wheat	88.43	18.06	13.25

Table 5. Apparent digestibility coefficient (ADC) of test ingredients on dry matter (DM) basis.

Ingredient	Dry Matter	Gross Energy	Crude Protein
Corn Gluten Meal	84.13	80.66	86.42
Canola Meal	67.61	76.90	88.14
Canola protein Concentrate	75.69	78.81	85.59
Fish meal	97.89	100.00	96.32
Soybean meal	69.24	79.28	95.10
Soy Protein Concentrate	73.71	78.39	93.36
Pea Meal	26.71	26.50	80.44
Pea protein concentrate	73.01	85.79	89.66
Wheat	21.97	43.57	84.28

Experiment 2

The replacement of fish meal with soybean meal in salmonid diets results in intestinal damage and reduced nutrient utilization when inclusion rates of greater than 20% SBM are used. Although this effect has been well established for SBM there has not been any examination of the properties of other plant ingredients used to replace fish meal. Corn gluten meal is a widely used plant protein ingredient in aquaculture and was therefore included in this study. We also examined wheat, pea protein concentrate and canola protein concentrate as promising Saskatchewan-produced ingredients. High-protein wheat is used as a pellet binder while PPC and CPC are protein sources.

Objective:

1) Determine the effect of feeding 0, 10, 20, 30, or 40% inclusion rates of wheat, corn gluten meal (CGM), soybean meal (SBM), pea protein concentrate (PPC), canola protein concentrate (CPC) on nutrient digestibility in rainbow trout.

Materials and methods

Fish Management
Rainbow trout were maintained in 350 L tanks that were part of a recirculating system using biological filtration. Water temperature was maintained at 15 ± 1° C. Dissolved oxygen, nitrate, nitrite, ammonia and pH were monitored regularly. Photoperiod was a 14 h light/10 h dark cycle. In all 6 digestibility experiments were performed using 5 diets per experiment. The formulation of the basal diet is shown in Table 1. The 10, 20, 30 and 40% inclusion rate diets were prepared by mixing the ingredient with the basal diet at 10:90, 20:80, 30:70 and 40:60 ratios respectively. Three replicates per treatment were used in all experiments. The fish were maintained in accordance with the guidelines of the Canadian Council on Animal Care (CCAC 1984).

Digestibility Determination

Apparent digestibility coefficients (ADC) were measured using an indirect method with diets containing 1% celite as a nonabsorbable indicator. The diets were cold extruded, dried in a forced air oven (55°C, 12 h), chopped and screened to obtain the appropriate pellet size. The fish were adapted to the experimental diets for five days and fecal material collected over a seven day period using a settling column which separated the fecal material from the effluent water. Following collection, feces were centrifuged (5000 X g, 15 min), frozen and freeze dried.

Laboratory Analysis
Experimental diets and fecal material were analyzed for moisture (AOAC 1990, method no. 934.01) and energy (oxygen bomb calorimetry; Parr Adiabatic Calorimeter, Model 1200). Nitrogen content was determined by combustion method (AOAC 1995). Protein was estimated by multiplying nitrogen content by 6.25. Determination of acid insoluble ash was performed according to the following procedure. Samples (quadruple replicates)

were charred (250°C, 18 h), followed by a gradual increase in temperature to 500°C (3 h), and then ashed (48 hours). Four mL of 4N HCl was added and the samples were heated at 120°C for a minimum of one hour. Samples were then centrifuged (3000 x g, 10 min) followed by aspiration of the supernatant. Five mL of water was added, vortexed, centrifuged and aspirated two times. The samples were then dried overnight (80°C) followed by ashing (500°C, 24 h).

Digestibility Calculations

Digestibility values were calculated as described above in Experiment 1.

Statistical Analyses

The effect of ingredient inclusion on digestibility was analysed using regression analysis procedure of SPSS (Version 14.0). A forward stepwise method was used to develop the regression model and ingredient effects in the final model were significant at the 5% level of significance.

Results

Table 6 and Figure 1 show the effects of ingredient inclusion rates on dry matter, energy and protein digestibility. Dry matter and energy digestibility decreased significantly with increasing inclusion rates of wheat and soybean meal ($P < 0.05$) but PPC, CPC, CGM and fish meal had no effects on energy or dry matter digestibility. There were no significant effects of ingredients on protein digestibility in the experiments.

Table 7 shows the regression coefficients for the ingredient effects on nutrient digestibility. For dry matter digestibility, only the coefficients for wheat (-0.006) and SBM (-0.003) were significant ($P < 0.05$). This indicates that a 10% inclusion of wheat would reduce dry matter digestibility by 0.06 units and 10% inclusion of SBM would decrease dry matter digestibility by 0.03 units. For energy digestibility, the coefficients for wheat (-0.005) and SBM (-0.003) were again the only significant ones. For protein digestibility, none of the ingredients had significant coefficients ($P > 0.05$). Based on these results we developed the digestibility prediction models based in Table 4. The dry matter and energy models are significant with r^2 values of 0.456 and 0.470 respectively. The prediction model for protein digestibility is not significant ($r^2 = 0.051$).

Table 6. Effect of ingredient inclusion rate on apparent digestibility coefficients of dry matter, energy and crude protein in rainbow trout.

	Ingredient Inclusion (%)					
Dry Matter	0	10	20	30	40	Pooled SEM
CPC	0.702	0.699	0.707	0.736	0.766	0.091
Fish meal	0.702	0.721	0.738	0.781	0.8	0.100
CGM	0.702	0.713	0.711	0.728	0.766	0.060
PPC	0.702	0.709	0.706	0.726	0.755	0.072
SBM	0.702	0.691	0.676	0.642	0.617	0.075
Wheat	0.702	0.615	0.609	0.556	0.582	0.343

Energy	0	10	20	30	40	Pooled SEM
CPC	0.769	0.739	0.736	0.782	0.816	0.034
Fish meal	0.769	0.777	0.786	0.834	0.849	0.020
CGM	0.769	0.768	0.766	0.788	0.808	0.019
PPC	0.769	0.756	0.757	0.782	0.799	0.107
SBM	0.769	0.769	0.707	0.734	0.680	0.051
Wheat	0.769	0.615	0.609	0.556	0.582	0.054

Protein	0	10	20	30	40	Pooled SEM
CPC	0.911	0.905	0.898	0.915	0.926	0.016
Fish meal	0.911	0.907	0.899	0.913	0.912	0.038
CGM	0.911	0.916	0.903	0.913	0.920	0.049
PPC	0.911	0.902	0.902	0.923	0.933	0.023
SBM	0.911	0.908	0.914	0.944	0.935	0.022
Wheat	0.911	0.903	0.908	0.924	0.937	0.016

Figure 1. Effect of ingredient inclusion rate on apparent digestibility coefficients of dry matter, energy and crude protein in rainbow trout.

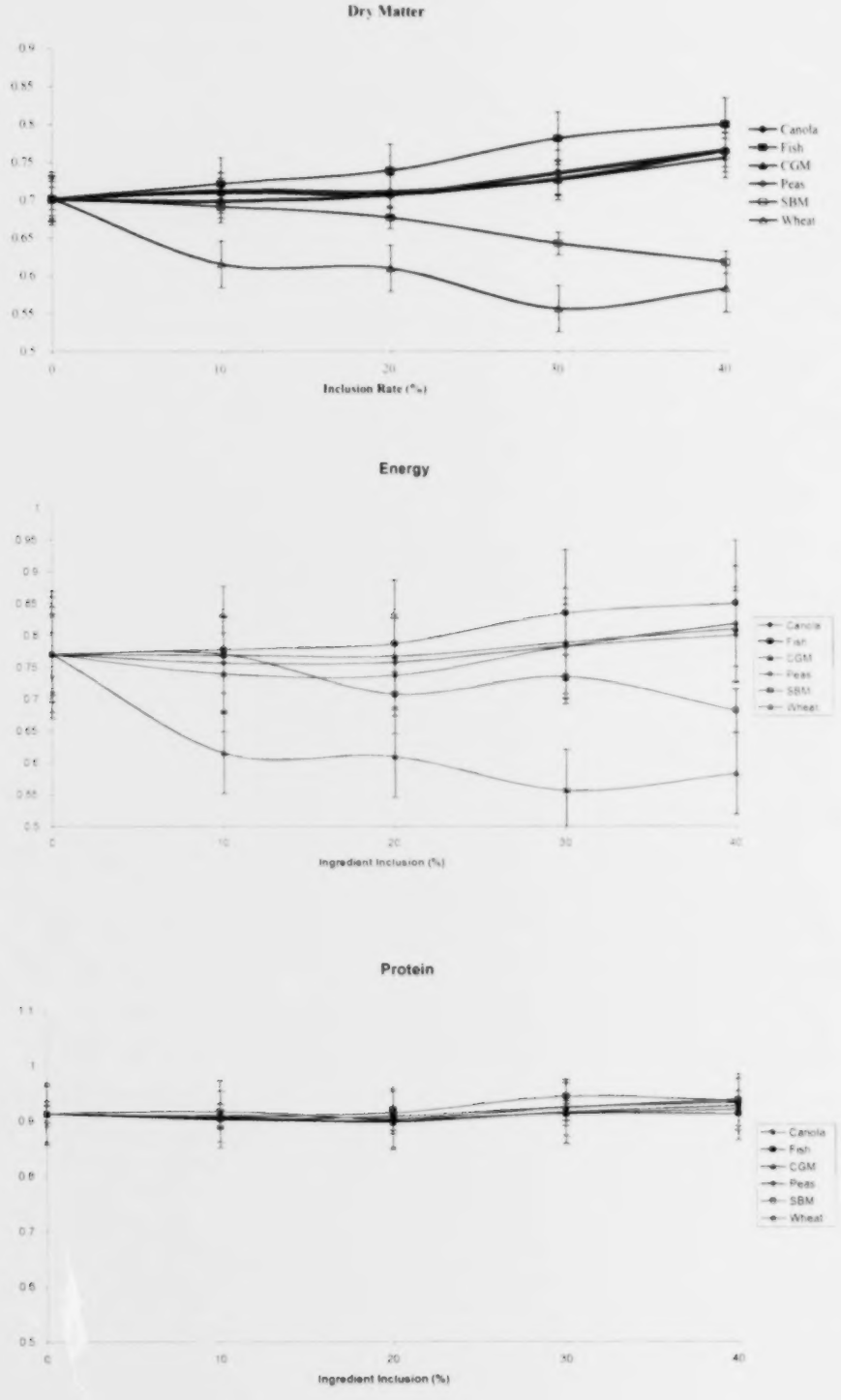


Table 7. Regression analysis of ingredient apparent digestibility coefficients for dry matter, energy and protein.

Dry Matter			
Ingredient	Coefficient	SEM	P-value
(Constant)	0.880	0.0887	< 0.01
Wheat	-0.006	0.0012	< 0.01
CGM	0.000	0.0012	0.769
SBM	-0.003	0.0011	< 0.01
Fish	0.001	0.0011	0.566
PPC	0.000	0.0011	0.785
CPC	0.000	0.0011	0.912
Energy			
	Coefficient	SEM	P-value
(Constant)	0.914	0.0733	< 0.01
Wheat	-0.005	0.0010	< 0.01
CGM	0.000	0.0010	0.754
SBM	-0.003	0.0009	< 0.01
Fish	0.001	0.0009	0.536
PPC	0.000	0.0009	0.622
CPC	0.000	0.0009	0.806
Protein			
	Coefficient	SEM	P-value
(Constant)	0.923	0.0055	< 0.01
Wheat	0.043	0.0006	0.968
CGM	-0.067	0.0004	0.955
SBM	-0.013	0.0003	0.978
Fish	0.000	0.0002	0.536
PPC	0.138	0.0003	0.968
CPC	0.011	0.0007	0.968

Conclusions

1) Soybean meal has significant negative effects on energy and dry matter digestibility and should be limited in diets fed to rainbow trout.
2) Wheat also has significant negative effects on energy and dry matter digestibility in rainbow trout and should be used at the minimum level that provides adequate pellet durability.
3) Canola protein concentrate and pea protein concentrate do not negatively affect dry matter, energy or protein digestibility in rainbow trout at inclusion levels up to 40%. This supports their use to replace fish meal.

Milestone 4. Growth Study I

Introduction

Work done in Milestone 3 showed that soybean meal and wheat alone among the 6 ingredients evaluated evaluated decreased the digestibility of feeds when included at up to 40% of the diet in rainbow trout. This suggests that Saskatchewan protein sources such as pea and canola might have a significant advantage over soybeans in salmonid diets. The objective of this study was to determine the effect of feeding pea, canola or soy products as meals or protein concentrates at graded levels up to 30% of the diet to determine the effect on growth performance of rainbow trout.

Materials and methods

Fish Management
Rainbow trout were maintained in 350 L tanks that were part of a recirculating system using biological filtration. Water temperature was maintained at 15 ± 1° C. Dissolved oxygen, nitrate, nitrite, ammonia and pH were monitored regularly. Photoperiod was a 14 h light/10 h dark cycle. There were a total of 6 growth studies in all testing pea meal, pea protein concentrate, canola meal, canola protein concentrate, soybean meal and soy protein concentrate at inclusion levels of 0, 7.5, 15, 22.5 and 30%. Diets were formulated to contain 4200 kcal/kg of DE and 38.6% digestible crude protein. All amino acids were balanced according to NRC, 1993 levels. Diet formulations are shown in Table 8.

Table 8. Diet formulations used in growth experiments to test the effect of inclusion rate of pea meal, pea protein concentrate, soybean meal, soy protein concentrate, canola meal and canola protein concentrate on the specific growth rate of rainbow trout.

Pea Meal

Ingredient Name	0	7.5	15	22.5	30
Fish Oil	141.3	145.1	148.8	152.6	156.3
L-Lysine HCl	0.0	0.0	0.0	0.0	0.0
DL-Methionine	0.8	0.6	0.4	0.2	0.0
Fish Meal	390.0	389.3	388.6	387.8	387.1
Corn Gluten meal	11.2	32.1	53.1	74.1	95.0
Wheat	100.0	86.9	73.8	60.7	47.6
Vit/Mineral Premix	10.0	10.0	10.0	10.0	10.0
Choline Cl	4.0	4.0	4.0	4.0	4.0
Meat and Bone Meal	224.8	168.6	112.4	56.2	0.0
Pea Meal	0.0	75.0	150.0	225.0	300.0
Solka Floc	117.9	88.5	59.0	29.5	0.0
	1000.0	1000.0	1000.0	1000.0	1000.0

	Pea Meal Diets				
Nutrient (%)	0	7.5	15	22.5	30
PHOS. TOTAL	2.07	1.85	1.64	1.42	1.20
DE Trout (MJ/kg)	17.58	17.58	17.58	17.58	17.58
Digestible Crude Protein	38.62	38.62	38.62	38.62	38.62
Digestible Methionine	1.08	1.08	1.09	1.09	1.09
Digestible Cysteine	0.36	0.38	0.39	0.41	0.42
Digestible Met+Cys	1.52	1.52	1.52	1.52	1.52
Digestible Lys	2.92	2.92	2.92	2.92	2.92
Digestible Thr	1.69	1.70	1.71	1.72	1.73
Digestible Arg	2.59	2.60	2.60	2.61	2.61
Digestible Ile	1.66	1.68	1.70	1.72	1.74
Digestible Val	2.15	2.15	2.15	2.14	2.14

Pea protein concentrate

Ingredient Name	0	7.5	15	22.5	30
Fish Oil	141.3	136.5	131.7	126.9	122.03
L-Lysine HCl	0.0	0.0	0.0	0.0	0
DL-Methionine	0.8	1.1	1.4	1.7	2
Fish Meal	390.0	361.4	332.7	304.1	275.41
Pea Protein Concentrate	0.0	75.0	150.0	225.0	300
Corn Gluten meal	11.2	24.0	36.9	49.8	62.7
Wheat	100.0	100.0	100.0	100.0	100
Vit/Mineral Premix	10.0	10.0	10.0	10.0	10
Choline Cl	4.0	4.0	4.0	4.0	4
Meat and Bone Meal	224.8	182.7	140.5	98.4	56.24
Solka Floc	117.9	105.4	92.8	80.2	67.63
	1000.0	1000.0	1000.0	1000.0	1000.0

	Pea Protein Concentrate Diets				
Nutrient (%)	**0**	**7.5**	**15**	**22.5**	**30**
PHOS. TOTAL	2.07	1.85	1.64	1.42	1.2
DE Trout (MJ/kg)	17.58	17.58	17.58	17.58	17.58
Digestible Crude Protein	38.62	38.62	38.62	38.62	38.62
Digestible Methionine	1.08	1.03	0.99	0.94	0.89
Digestible Cysteine	0.36	0.38	0.40	0.41	0.43
Digestible Met+Cys	1.52	1.52	1.52	1.52	1.52
Digestible Lys	2.92	2.92	2.92	2.92	2.92
Digestible Thr	1.69	1.68	1.68	1.67	1.66
Digestible Arg	2.59	2.66	2.73	2.80	2.87
Digestible Ile	1.66	1.67	1.68	1.69	1.7
	2.15	2.13	2.12	2.10	2.08

Soybean Meal

Ingredient Name	0	7.5	15	22.5	30
Fish Oil	141.3	142.6	143.9	145.2	146.54
L-Lysine HCl	0.0	0.0	0.0	0.0	0
DL-Methionine	0.8	0.9	1.0	1.1	1.17
Fish Meal	390.0	367.4	344.8	322.2	299.64
Soybean Meal	0.0	75.0	150.0	225.0	300
Corn Gluten meal	11.2	9.0	6.9	4.8	2.63
Wheat	100.0	100.0	100.0	100.0	100
Vit/Mineral Premix	10.0	10.0	10.0	10.0	10
Choline Cl	4.0	4.0	4.0	4.0	4
Meat and Bone Meal	224.8	180.1	135.3	90.6	45.83
Solka Floc	117.9	111.0	104.1	97.1	90.19
	1000.0	1000.0	1000.0	1000.0	1000.0

	0	7.5	15	22.5	30
Nutrient (%)	2.07	1.80	1.54	1.27	1
PHOS. TOTAL	17.58	17.58	17.58	17.58	17.58
DE Trout (MJ/kg)	38.62	38.62	38.62	38.62	38.62
Digestible Crude Protein	1.08	1.05	1.01	0.98	0.94
Digestible Methionine	0.36	0.39	0.41	0.44	0.46
Digestible Cysteine	1.52	1.52	1.52	1.52	1.52
Digestible Met+Cys	2.92	2.92	2.92	2.92	2.92
Digestible Lys	1.69	1.69	1.70	1.70	1.7
Digestible Thr	2.59	2.63	2.66	2.70	2.73
Digestible Arg	1.66	1.69	1.72	1.75	1.78
Digestible Ile	2.15	2.14	2.13	2.12	2.11

Soy Protein Concentrate

Ingredient Name	0	7.5	15	22.5	30
Fish Oil	141.3	150.5	159.7	168.9	178.05
L-Lysine HCl	0.0	0.6	1.3	1.9	2.54
DL-Methionine	0.8	1.3	1.8	2.3	2.86
Fish Meal	390.0	338.3	286.5	234.8	183.07
Soy Protein Concentrate	0.0	75.0	150.0	225.0	300
Corn Gluten meal	11.2	8.4	5.6	2.8	0
Wheat	100.0	100.0	100.0	100.0	100
Vit/Mineral Premix	10.0	10.0	10.0	10.0	10
Choline Cl	4.0	4.0	4.0	4.0	4
Meat and Bone Meal	224.8	195.5	166.2	137.0	107.69
Solka Floc	117.9	116.4	114.9	113.3	111.79
	1000.0	1000.0	1000.0	1000.0	1000.0

	Soy Protein Concentrate Diets				
Nutrient (%)	0	7.5	15	22.5	30
PHOS. TOTAL	2.07	1.80	1.54	1.27	1
DE Trout (MJ/kg)	17.58	17.58	17.58	17.58	17.58
Digestible Crude Protein	38.62	38.62	38.62	38.62	38.62
Digestible Methionine	1.08	1.01	0.93	0.86	0.78
Digestible Cysteine	0.36	0.38	0.41	0.43	0.45
Digestible Met+Cys	1.52	1.52	1.52	1.52	1.52
Digestible Lys	2.92	2.92	2.92	2.92	2.92
Digestible Thr	1.69	1.67	1.64	1.62	1.59
Digestible Arg	2.59	2.64	2.70	2.75	2.8
Digestible Ile	1.66	1.68	1.70	1.72	1.74
	2.15	2.12	2.10	2.07	2.04

Canola Meal

Ingredient (g kg^{-1})	0% CM	7.5% CM	15% CM	22.5% CM	30% CM
Fish oil	120.50	111.28	102.05	92.83	83.60
L-Lysine	1.90	2.73	3.55	4.38	5.20
DL-Methionine	1.80	1.40	1.00	0.60	0.20
Fish meal	400.00	413.20	426.40	439.60	452.80
Canola meal	0.00	75.00	150.00	225.00	300.00
Wheat flour	100.00	100.00	100.00	100.00	100.00
Vitamin premix	4.75	4.75	4.75	4.75	4.75
Mineral premix	4.75	4.75	4.75	4.75	4.75
Vitamin C	0.50	0.50	0.50	0.50	0.50
Choline chloride	4.00	4.00	4.00	4.00	4.00
Meat and bone meal	304.00	228.00	152.00	76.00	0.00
Solkafloc	57.80	54.40	51.00	47.60	44.20

Digestible nutrient (%)	0% CM	7.5% CM	15% CM	22.5% CM	30% CM
Phosphorus	2.46	2.14	1.82	1.49	1.17
DE (MJ/kg)	17.58	17.58	17.58	17.58	17.58
Crude Protein	38.62	38.62	38.62	38.62	38.62
Methionine	0.99	0.91	0.83	0.75	0.67
Cysteine	0.34	0.37	0.40	0.42	0.45
Methioning and Cysteine	1.52	1.52	1.52	1.52	1.52
Lysine	2.92	2.92	2.92	2.92	2.92
Threonine	1.61	1.61	1.62	1.62	1.62
Arginine	2.57	2.53	2.49	2.44	2.4
Isoleucine	1.57	1.57	1.58	1.58	1.58
Valine	2.07	2.03	2.00	1.96	1.92

Canola Protein concentrate

Ingredient Name	0	7.5	15	22.5	30
Fish Oil	141.3	150.5	159.7	168.9	178.05
L-Lysine HCl	0.0	0.6	1.3	1.9	2.54
DL-Methionine	0.8	1.3	1.8	2.3	2.86
Fish Meal	390.0	338.3	286.5	234.8	183.07
Canola Protein Concentrate	0.0	75.0	150.0	225.0	300
Corn Gluten meal	11.2	8.4	5.6	2.8	0
Wheat	100.0	100.0	100.0	100.0	100
Vit/Mineral Premix	10.0	10.0	10.0	10.0	10
Choline Cl	4.0	4.0	4.0	4.0	4
Meat and Bone Meal	224.8	195.5	166.2	137.0	107.69
Solka Floc	117.9	116.4	114.9	113.3	111.79
	1000.0	1000.0	1000.0	1000.0	1000.0

	Soy Protein Concentrate Diets				
Nutrient (%)	0	7.5	15	22.5	30
PHOS. TOTAL	2.07	1.80	1.54	1.27	1
DE Trout (MJ/kg)	17.58	17.58	17.58	17.58	17.58
Digestible Crude Protein	38.62	38.62	38.62	38.62	38.62
Digestible Methionine	1.08	1.01	0.93	0.86	0.78
Digestible Cysteine	0.36	0.38	0.41	0.43	0.45
Digestible Met+Cys	1.52	1.52	1.52	1.52	1.52
Digestible Lys	2.92	2.92	2.92	2.92	2.92
Digestible Thr	1.69	1.67	1.64	1.62	1.59
Digestible Arg	2.59	2.64	2.70	2.75	2.8
Digestible Ile	1.66	1.68	1.70	1.72	1.74
	2.15	2.12	2.10	2.07	2.04

Growth Study

Each of the 6 growth studies consisted of 5 levels of each ingredient: 0, 7.5, 15, 22.5 and 30%. Three tanks of rainbow trout were assigned to each inclusion level and fish were fed the diets twice daily to satiety for a period of 12 weeks. Fish weight was taken at the start and end of the experiment and feed intake was measured daily. Growth was assessed using specific growth rate; a logarithmic function that best models the growth of salmonid fish.

Statistical analysis

The results of each trial were analyzed by regression. Linear and quadratic equations between inclusion rate of each ingredient and the specific growth rate of rainbow trout were fitted to the data using the regression procedure of SPSS. The values for b_0 (the y intercept) b_1 (coefficient for x) and b_2 (coefficient for x^2) were calculated. Regressions were considered significant when $P < 0.05$.

Results

Table 9 shows the mean specific growth rate for all inclusion levels of the 6 ingredients tested.

1) There were no significant differences between the means for pea meal, pea protein concentrate, soy protein concentrate or canola protein concentrate ($P > 0.05$).
2) The specific growth rates of trout fed soybean meal and canola meal showed a significant decrease at the 30% inclusion rate compared with the controls.

Table 10 shows the linear and quadratic regression parameters between ingredient inclusion rate and specific growth rate for all 6 ingredients tested.

1) There was a significant positive quadratic relationship between the inclusion rate of pea meal and the specific growth rate of rainbow trout (Figure 2). The specific growth rates tended to increase at higher inclusion rates. This indicates that pea meal can be added to rainbow trout diets at up to 30% with no negative impact on fish growth.
2) There was no significant linear or quadratic relationship between the inclusion of pea protein concentrate and specific growth rate of rainbow trout (Figure 3). This indicates that pea protein concentrate can be fed at levels up to 30% with no negative impact of fish growth.
3) There was a significant negative quadratic relationship between the inclusion rate of soybean meal and the specific growth rate (Figure 4). Growth rate was reduced to 95% of controls at 18.6% inclusion and to 90% of controls at 20.5% inclusion. This indicates that soybean meal levels should be limited in rainbow trout diets.
4) There was a significant negative linear effect between soy protein concentrate and specific growth rate (Figure 5). Growth rate was reduced to 95% of controls at 7.8% inclusion rate and 90% of controls at 15.6% inclusion rate.

5) There was a significant negative linear relationship between the inclusion rate of canola meal and the specific growth rate of the trout (Figure 6). Growth rate was reduced to 95% of the controls at 4.5% inclusion rate and 90% of controls at 15.6% inclusion rate.
6) There was a significant positive quadratic relationship between the inclusion rate of canola protein concentrate and the specific growth rate of trout (Figure 7). At levels up to 30% inclusion the specific growth rate was increased by the addition of canola protein concentrate.

The overall conclusion is that the inclusion rates of soybean meal, soy protein concentrate and canola meal should be limited in diets fed to rainbow trout. Pea meal, pea protein concentrate and canola protein concentrate do not negatively impact the growth of rainbow trout at levels up to 30% of the diet.

Table 9. Specific growth rate (%/d) of fish fed 6 test ingredients at increasing inclusion rates.

Inclusion Rate (%)	Pea Meal	PPC	Soybean Meal	SPC	Canola Meal	CPC
0.0	1.62	1.42	0.55b	0.74	0.73a	0.80
7.5	1.41	1.30	0.62b	0.72	0.63ab	0.85
15.0	1.32	1.33	0.67b	0.71	0.63ab	0.89
22.5	1.39	1.29	0.54b	0.55	0.49ab	0.91
30.0	1.53	1.30	0.32a	0.60	0.48b	0.86
SEM	0.051	0.019	0.039	0.034	0.038	0.036

abMeans with different labels within columns are significantly different ($P < 0.05$)

Table 10. Linear and quadratic regression parameters of the relation between ingredient inclusion rate and specific growth rate of rainbow trout.

	Unstandardized Coefficients				
	Constant	Inclusion	Inclusion^2	r^2	*P*-value
Pea Meal					
Linear	1.491	-0.003		0.065	0.679
Quadratic	1.614	-0.035	0.0010	0.990	0.010
Pea Protein Concentrate					
Linear	1.377	-0.003		0.525	0.166
Quadratic	1.406	-0.011	0.0003	0.766	0.234
Soybean Meal					
Linear	0.703	-0.010		0.665	0.093
Quadratic	0.625	0.011	-0.0007	0.665	0.047
Soy Protein Concentrate					
Linear	0.749	-0.005		0.909	0.012
Quadratic	0.738	-0.002	-0.0001	0.935	0.065
Canola Meal					
Linear	0.720	-0.008		0.914	0.011
Quadratic	0.723	-0.009	-0.00003	0.915	0.085
Canola Protein Concentrate					
Linear	0.825	0.002		0.467	0.204
Quadratic	0.793	0.011	-0.0003	0.965	0.035

Figure 2. Relationship between inclusion rate of pea meal and specific growth rate in rainbow trout

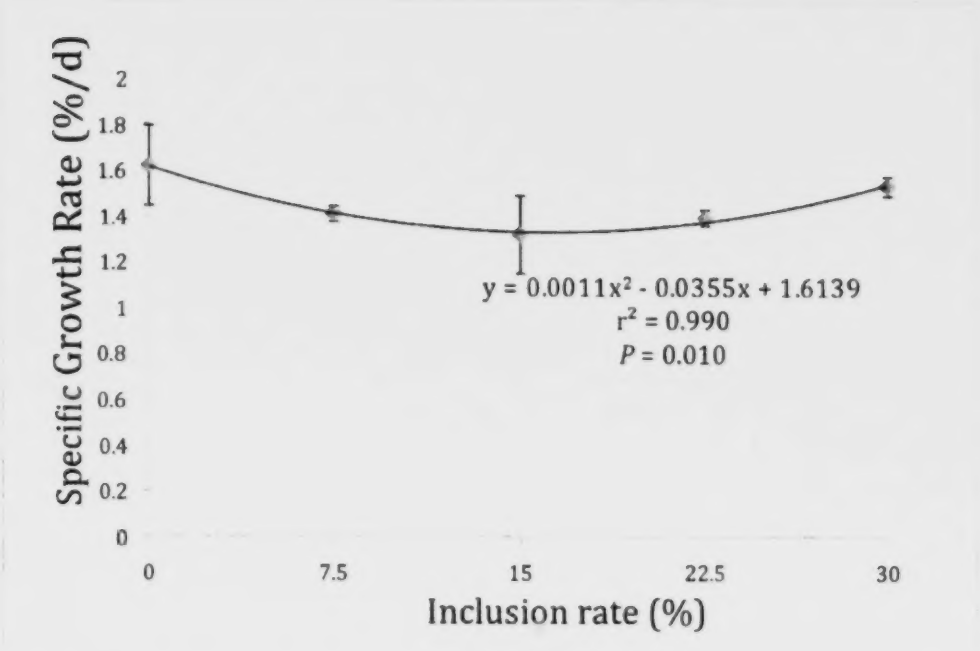


Figure 3. Relationship between inclusion rate of pea protein concentrate and specific growth rate in rainbow trout

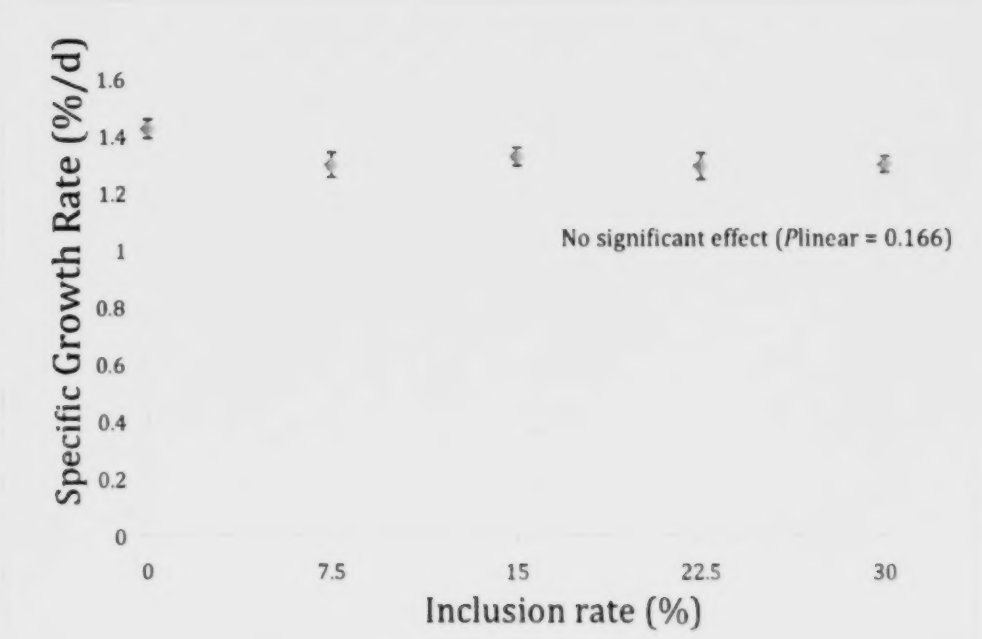


Figure 4. Relationship between inclusion rate of soybean meal and specific growth rate in rainbow trout

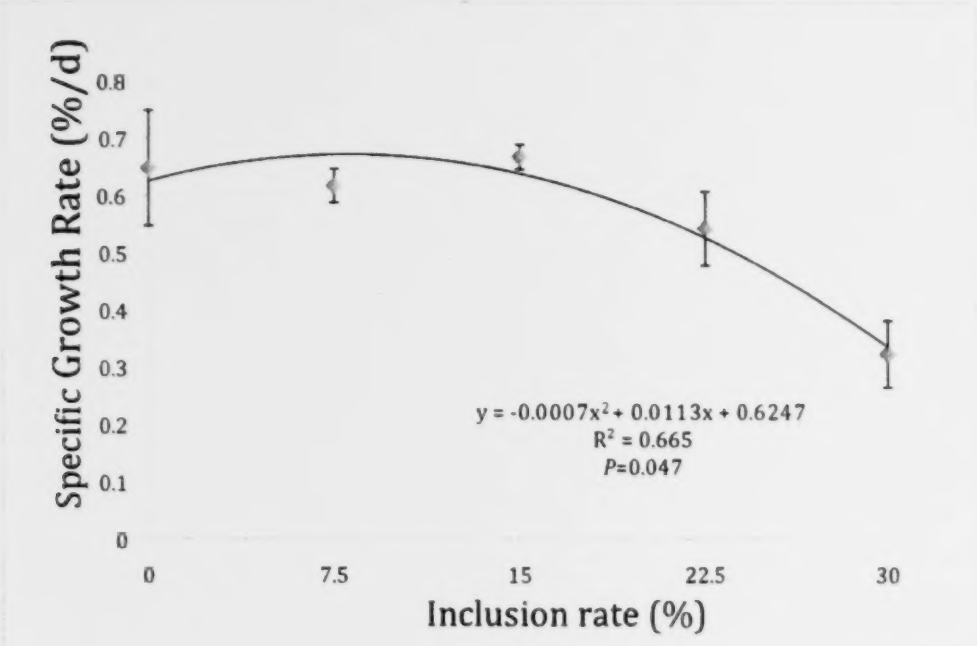


Figure 5. Relationship between inclusion rate of soy protein concentrate and specific growth rate in rainbow trout

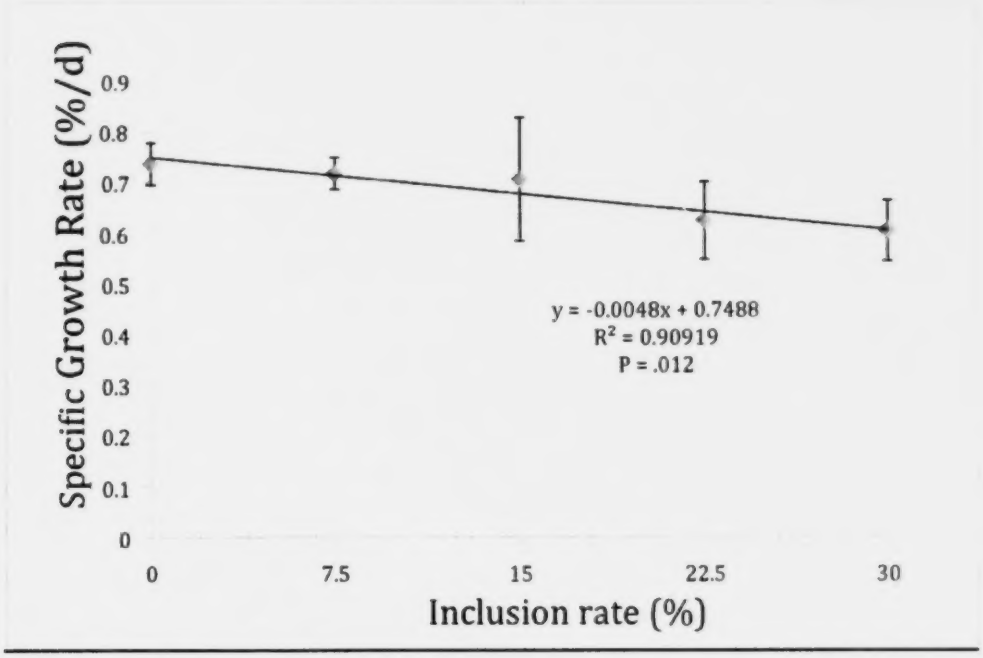


Figure 6. Relationship between inclusion rate of canola meal and specific growth rate in rainbow trout

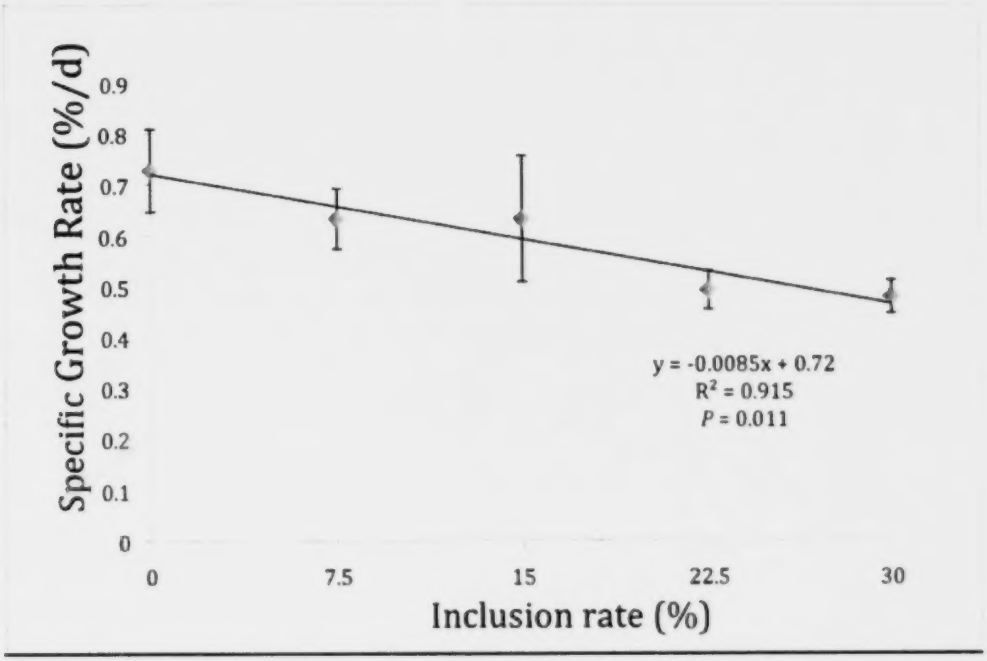
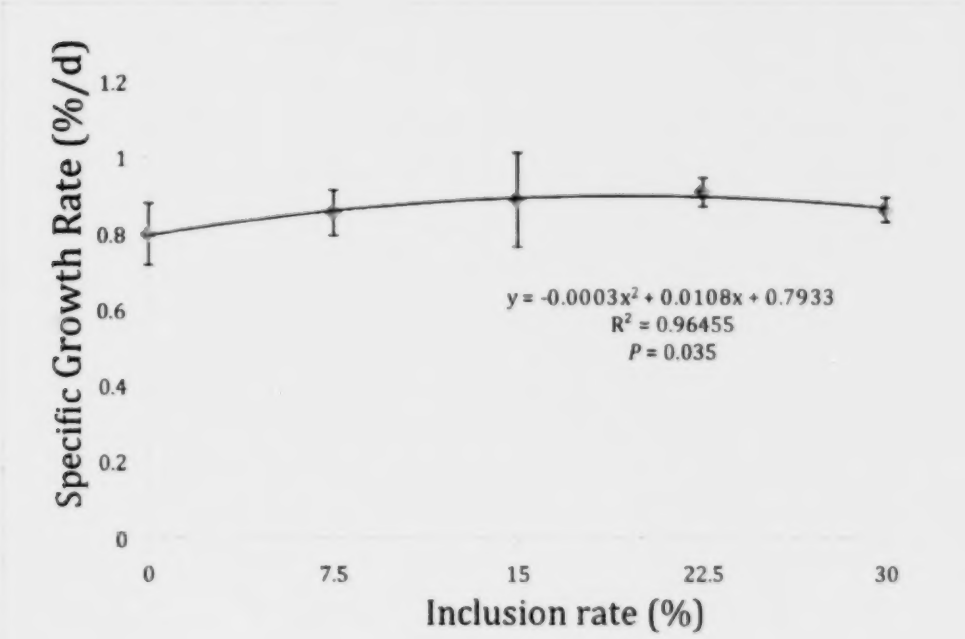


Figure 7. Relationship between inclusion rate of canola protein concentrate and specific growth rate in rainbow trout



Conclusions

Soybean meal is the most well studied vegetable protein used to replace fish meal in salmonid diets. It is well known that inclusion rates of soybean meal above 20% in the diet will lead to decrease growth and intestinal inflammation in salmon and trout. It was naturally assumed that this was true for all plant protein sources. However, these studies have demonstrated that not all ingredients have the same effect on salmonid fish. Pea meal and pea and canola protein concentrates do not have any negative effects on fish growth at levels up to 30% of the diet or nutrient digestibility at levels of up to 40%. This advantage provides these products with a significant advantage over soybeans in salmonid diets. With the start of commercial manufacturing of canola protein concentrate by MCN Bioproducts in Arborfield SK and the existing capacity for the production of pea meal and pea protein concentrate in Saskatchewan. The opportunity exists to markedly increase the use of these products in aquafeeds in Canada and Internationally. We will be presenting this information at the European Aquaculture Conference in Trondheim Norway in August 2009 and are preparing a manuscript for publication in the Journal Aquaculture.

Other

This work will form part of the research section of a Ph.D. thesis by Stephanie Nilson.

Publications

Collins S.A. and Drew, M.D. 2007. Opportunities for Western Canadian pulse crops in aquafeeds. Proceedings of the 24th Annual meeting of the Aquaculture Association of Canada. September 23-26, 2007. Edmonton AB. Published on CD.

S.A. Nilson, A.G. Van Kessel, J.E. Hill, M.D. Drew. Growth of rainbow trout fed pea meal and pea protein concentrate at increasing inclusion levels. Proceedings of the European Aquaculture Meeting. August 14-17, 2009. Trondheim Norway.

Extension activities

We have been invited to present these results at the meeting of the Canada, Norway, US Trilateral Research Group for Finfish Aquaculture this August in Trondheim Norway.

Expense Statement

A financial statement will be sent by research services.